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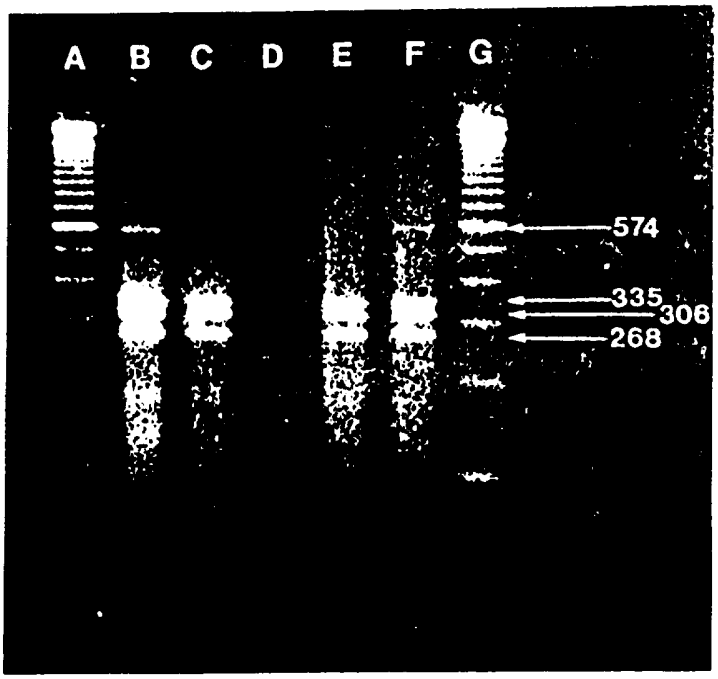
(54) Title: NOVEL ALLELE OF HUMAN HISTAMINE H_2 RECEPTOR AND METHODS OF DETECTION OF H_2 RECEPTOR VARIANTS

(57) Abstract

A nucleotide sequence coding for a region of a human histamine H_2 receptor, comprising one or more of the following base substitutions compared with the published sequence in Gantz et al (1991) Biochem Biophys Res Comm 178, 3, 1386 - 1392, and from which the positional notation is taken: site of change - base: 398 - C, 525 - T, 620 - G, 649 - G, 692 - G, 802 - A. Also included are oligonucleotides suitale for use as primers for the amplification or sequencing of DNA corresponding to a specific region of a human histamine H_2 receptor, and a diagnostic kit comprising at least one of these oligonucleotides.



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NOVEL ALLELE OF HUMAN HISTAMINE H_2 RECEPTOR AND METHODS OF DETECTION OF H_2 RECEPTOR VARIANTS

Technical Field

This invention relates to the detection of variations in human histamine H_2 receptors, and more particularly to the development of new compounds useful in the sequencing and identification of a human histamine H_2 receptor and their use in the diagnosis and treatment of certain human disorders, for example, brain disorders. The invention also relates to new compounds and a method for detecting an allelic polymorphic variation within the human population for the gene encoding the histamine H_2 receptor and their use in the diagnosis and treatment of human disorders.

Background Art

The human H_2 receptor was first identified by Black et al Nature (1972), 236, 385 - 390. This was followed by the demonstration of the receptor in the mammalian brain by Baudry et al (1975) Nature 253, 362 - 363, and Haas and Bucher (1975) Nature 255, 634 - 635. Gantz et al (1991) Biochem. Biophys. Res. Comm. 178,3, 1386-1392 have recently identified the sequence of a human H_2 receptor cDNA from gastric parietal cells by using the

polymerase chain reaction (PCR) and degenerated oligonucleotide primers whose sequence was obtained from the canine H_2 receptor previously cloned by this group, Gantz et al (1991) Proc. Nat. Acad. Sci. USA 88, 429 - 433. This sequence was characterised as an intronless gene encoding a typical seven transmembrane domain aminergic receptor protein.

The receptor is coupled to heterotrimeric GTPases (G proteins), but differs from other monoamine receptors in this G protein coupled superfamily in several respects. The human gastric H_2 receptor is shorter than most other receptors in this class (359 amino acids) and lacks the two serine residues in the fifth transmembrane region (TM5). There exists instead an aspartate and a threonine residue, so far unique in this region. These two residues may be important for binding with the nitrogen atoms of the imidazole ring of histamine as suggested by Birdsall (1991) Trends in Pharmacological Sci. Jan, 12, 9 - 10.

Histamine is a natural constituent of many organs and tissues including the gastrointestinal tract, the immune system and the brain, Green et al (1987) Agents and Actions 22, 1 - 15. It is a central neurotransmitter in the brain and is formed in the posterior hypothalamus from exogenous histidine by histidine decarboxylase (HDC). It is subsequently metabolised by histamine

methyltransferase (HMT), Prell et al (1986) Ann. Rev. Neurosci. 9, 209 - 254. The cell bodies and neuronal pathways for histamine have been mapped in the human brain using immunocytochemistry by Panula et al (1990) Neuroscience 34, 127 - 132. Its cells project from the tuberomamillary nucleus of the posterior hypothalamus to almost every region of the brain. There are three known histamine receptors; H_1, H_2 and H_3, the latter functioning as an autoreceptor. The H_2 receptor specifically has been localised in the human brain by Traiffort et al (1992) J. of Neurochem. 59, 1, 290 - 299. using receptor autoradiography.

Histamine is known to have significant effects in the central nervous system (CNS). It has been implicated in the CNS mediated mechanisms of arousal ever since the sedating effect of H_1 receptor antagonists (eg,. chlorpheniramine, chloropromazine) had been noticed clinically. The use of H_2 receptor antagonists in the human brain however, has shown, that these compounds, unlike those acting on the H_1 receptor, do not produce any effect on psychomotor functioning, or a subjective feeling of sedation or arousal in healthy subjects, White et al (1988) Psychopharmacology 95, 1 - 14. Some H_2 receptor antagonists (eg. cimetidine) are known to cause confusion in elderly or severely medically ill patients, perhaps in part due to a co-existing anti-cholinergic effect. H_1 and H_2 receptor antagonists in large doses

have been reported to cause hallucinations, Csillag et al (1973) Med. J. Aust. 1, 653 - 654, Argawal (1978) J. Am. Med. Assoc. 240, 214. Animal studies have shown that histamine applied directly to the hippocampus, where there is the highest level of activity of the H_2 receptor, will induce psychomotor withdrawal and decreased exploratory behaviour. The above evidence has led to the conclusion that H_1 receptor systems are excitatory in the terms of arousal and motivated behaviour whilst H_2 receptor systems are inhibitory in this respect, Alvarez and Banzan (1985) Physiol. and Beh. 34, 661 - 664 and (1986) Physiol. and Beh. 37, 39 - 45, White et al (1988) supra.

The H_2 receptor is a site of action of various compounds used in the treatment of psychiatric disorders eg. amitriptyline and mianserin, Traiffort et al (1992) supra. Kaminsky et al (1990) The Lancet 335, 1351 - 1352 and (1991) Schizophrenia Bull. 4, 318 - 319 have reported the successful response of patients with chronic, predominantly negative type schizophrenia, to the highly specific H_2 receptor antagonist famotidine. For example in one patient there was a substantial amelioration of the deficit symptoms of schizophrenia (eg apathy, social withdrawal, and blunted affect) while on famotidine, relapse in these symptoms on withdrawal, and improvement on re-institution of this drug, Kaminski, US Patents Nos. 5070101 and 5177081. Prell et al (1992) Abstract, part

1, 199.6 Soc. for Neurosci. Annual Meeting, Anaheim Cal. have shown substantially raised levels of N-tele-methyl histamine, a metabolite of histamine in the cerebrospinal fluid of patients with schizophrenia which correlates with those patients with the occurrence of negative symptoms of this disorder assessed using the Psychiatric Symptoms Assessment Scale. These levels were not significantly different between patients free from medication and those on neuroleptic therapy. It is therefore postulated that there is an increase in histaminergic activity in patients with chronic schizophrenia.

The disclosures of all the above mentioned publications are incorporated herein by reference for all purposes.

Additionally, histamine, acting via its receptors, including the H_2 receptor, is believed to be critically involved in a number of diseases of organs other than the brain; these include peptic ulceration, allergic reactions, including asthma, immune-mediated disorders, and possibly some tumours.

The histamine H_2 receptor is one of many receptors in the body. Compounds used to treat many diseases work by activating a receptor or inhibiting the action if its natural ligand. Variations in receptors amongst the

population are known to be caused by allelic variation and this variation, can alter the response of a disease to a drug amongst patients. An example of this would be the response to clozapine, used to treat, schizophrenia associated with allelic variation in the 5-HT_{2A} receptor demonstrated by Arranz el al (1995) Lancet, 346(8970), 281-282.

Disclosure of the Invention

The present invention is concerned in one aspect with improvements in the diagnosis and/or treatment of human neurological and psychiatric disorders, and more particularly in the diagnosis and treatment of schizophrenia. In another aspect, the invention is concerned with improvements in the diagnosis and/or treatment of diseases of other systems or organs of the human body.

As a first step to the present invention, the Applicants devised a new oligonucleotide probe to the human H_2 receptor mRNA in accordance with the published cDNA sequence available for the gastric parietal cell. Surprisingly, studies using this probe with in-situ hybridization histochemistry on human post-mortem brain tissue produced evidence of a mismatch in the nucleotide sequence for the brain H_2 receptor and the sequence for the gastric parietal cell H_2 receptor. This discovery was

made by recording melt-curve estimations for the optimum hybridization incubation temperature using the method of Davis et al (1986) "Basic Methods in Molecular Biology" page 77 Elsevier Science Publishing Co. It was found that the sequence mismatch is of the order of 10%.

It was apparent, therefore, that there is a hitherto unrecognised allele or subtype of the human histamine H_2 receptor gene, which may be specific to the brain.

In a first aspect, the invention provides a sequence for a novel allele of a human histamine H_2 receptor gene comprising up to six single base substitutions compared with the cDNA sequence published by Gantz et al (1991) Biochem Biophys Res Comm 178,3,1386-1392 as follows:

site of change	base change	amino acid alteration
398	T - C	Val - Gly
525	A - T	Lys - Asn
620	A - G	Lys - Asp
649	A - G	Asn - Asp
692	A - G	Lys - Arg
802	G - A	Val - Met

In another aspect, the invention provides a nucleotide sequence coding for a region of a human histamine H_2 receptor, comprising one or more of the following base substitutions compared with the published

sequence in Gantz et al (1991) supra, and from which the positional notation is taken:

site of change	base
398	C
525	T
620	G
649	G
692	G
802	A

The nucleotide sequence of the invention can, for example, comprise the following sequence (as also listed in SEQ ID NO: 1):

5'

CAGCTCGGGTCGCCATCTCTCTGGTCTTAATTTGGGTCATCTCCATTACCCTGTC
CTTTCTGTCTATCCACCTGGGGTGGAACAGCAGGAACGAGACCAGCAAGGGCAAT
CATACCACCTCTAAGTGCAATGTCCAGGTCAATGAAGTGTACGGGCTGGTGGATG
GGCTGGTCACCTTCTACCTCCCGCTACTGATCATGTGCATCACCTACTACCGCAT
CTTCAGGGTCGCCCGGGATCAGGCCAAGAGGATCGATCACATTAGCTCCTGGAAG
GCAGCCACCATCAGGGAGCACAGAGCCACAGTGACACTGGCCGCCGTCATGGGGG
CCTTCATCATCTGCTGGTTTCCCTACTTCACCGCGTTTGTGTACCGTGGGCTGAG
AGGGGATGATGCCATCAATGAGATGTTA 3'

As a specific exemplification, the nucleotide sequence of the invention can comprise the following sequence (as also listed in SEQ ID NO: 2):

5'

CCAATGGCACAGCCTCTTCCTTTTGCCTGGACTCTACCGCATGCAAGATCACCAT

CACCGTGGTCCTTGCGGTCCTCATCCTCATCACCGTTGCTGGCAATGTGGTCGTC

TGTCTGGCCGTGGGCTTGAACCGCCGGCTCCGCAACCTGACCAATTGTTTCATCG

TGTCCTTGGCTATCACTGACCTGCTCCTCGGCCTCCTGGTGCTGCCCTTCTCTGC

CATCTACCAGCTGTCCTGCAAGTGGAGCTTTGGCAAGGTCTTCTGCAATATCTAC

ACCAGCCTGGATGTGATGCTCTGCACAGCCTCCATTCTTAACCTCTTCATGATCA

GCCTCGACCGGTACTGCGCTGTCATGGACCCACTGCGGTACCCTGTGCTGGTCAC

CCCAGCTCGGGTCGCCATCTCTCTGGTCTTAATTTGGGTCATCTCCATTACCCTG

TCCTTTCTGTCTATCCACCTGGGGTGGAACAGCAGGAACGAGACCAGCAAGGGCA

ATCATACCACCTCTAAGTGCAATGTCCAGGTCAATGAAGTGTACGGGCTGGTGGA

TGGGCTGGTCACCTTCTACCTCCCGCTACTGATCATGTGCATCACCTACTACCGC

ATCTTCAGGGTCGCCCGGGATCAGGCCAAGAGGATCGATCACATTAGCTCCTGGA

AGGCAGCCACCATCAGGGAGCACAGAGCCACAGTGACACTGGCCGCCGTCATGGG

GGCCTTCATCATCTGCTGGTTTCCCTACTTCACCGCGTTTGTGTACCGTGGGCTG

AGAGGGGATGATGCCATCAATGAGATGTTAGAAGCCATCGTTCTGTGGCTGGGCT

ATGCCAACTCAGCCCTGAACCCCATCCTGTATGCTGCGCTGAACAGAGACTTCCG

CACCGGGTACCAACAGCTCTTCTGCTGCAGGCTGGCCAACCGCAACTCCCACAAA

ACTTCTCTGAGGTCCAACGCCTCTCAGCTGTCCAGGACCCAAAGCCGAGAACCCA

GGCAACAGGAAGAGAAACCCCTGAAGCTCCAGGTGTGGAGTGGGACAGAAGTCACG

3'

In another aspect of the invention, a series of new oligonucleotide primers have been developed for the identification of sequences in a sample comprising a human histamine H_2 receptor DNA, cDNA or RNA originating from a tissue sample or body fluid.

In this aspect, the invention provides new oligonucleotides, suitable for use as primers for the amplification of DNA corresponding to a region of a human histamine H_2 receptor, having nucleotide sequences selected from:

1) 5' CCAATGGCACAGCCTCTT 3' (as listed in SEQ ID NO: 3)
2) 5' CGTGACTTCTGTCCCACT 3' (as listed in SEQ ID NO: 4)
3) 5' CCAGGCAACAGGAAGAGA 3' (as listed in SEQ ID NO: 5)
4) 5' TCTCTTCCTGTTGCCTGG 3' (as listed in SEQ ID NO: 6)
5) 5' GCAGCAGAAGAGCTGTTG 3' (as listed in SEQ ID NO: 7)
6) 5' TCCAGGTCAATGAAGTGT 3' (as listed in SEQ ID NO: 8)
7) 5' ACACTTCATTGACCTGGA 3' (as listed in SEQ ID NO: 9)
8) 5' CCAAGAGGATCAATCACA 3' (as listed in SEQ ID NO: 10)
9) 5' TGTGATTGATCCTCTTGG 3' (as listed in SEQ ID NO: 11)

and a diagnostic kit comprising one or more of the new oligonucleotides.

The direction and base start numbers for the novel oligonucleotide primers are as follows:

Primer		Base Start No.
1)	Upstream	8
2)	Downstream	1036 and 1095
3)	Upstream	995
4)	Downstream	1012 (no. 3) in reverse)
5)	Downstream	898 and 1171

6)	Upstream	527
7)	Downstream	544 (no. 6) in reverse)
8)	Upstream	638
9)	Downstream	655 (no. 8) in reverse)

Information on the human histamine H_2 receptor was obtained from the MRC Daresbury database accessing "Genem 61" File no. M64799 - Human histamine H_2 receptor gene.

The above mentioned substitutions alter and in some instances introduce or remove new sites for cleavage by specific restriction endonucleases as follows:

base change site	**alters restriction map of:**
398	AluI, AvaI, BspWI, BsrI, CviJI
525	
620	Eco57
649	ClaI, Sau3A, TaqI
692	
802	MnlI

The invention further provides a diagnostic kit comprising one or more of the new oligonucleotide primers and, preferably, one or more of the above mentioned endonucleases, optionally with one or more buffers.

A kit may be used to establish genotype or base variations. This information may be used in predicting an individuals disease susceptibility, disease course,

prognosis and/or response to treatment as would be understood by those skilled in the art from the disclosure contained herein. The treatment response or efficacy which may be predicted may include drug treatment such as for example, use of H_2 receptor antagonist like famotidine or other forms of treatment such as social or psychological intervention.

Eucaryotic expression vectors comprising a DNA sequence coding for a protein and.or a peptide according to the invention are new materials and are also included in the invention. Host cells, for example, cloned human cell lines, such as NTera 2 c.d1, can be transformed using the new expression vectors and are also included in the invention.

Expression vectors and host cells transformed thereby, in accordance with the invention, can be prepared, for example, as detailed below, and the encoded protein studied, by one or more of the following exemplary methods:

1. Total RNA is extracted from homogenised human tissue, eg. brain, by the acid guanidine thiocyanate method (Chomczynski & Saach (1987), anal. Biochem. 161, 156-159). Messenger RNA (mRNA) is purified from this by hybridisation of oligo(d)T to the polyadenylated tails present on the majority of

mRNA's, for example, using the Promega PolyAttract ® system. Reverse transcription of the mRNA using specific reverse transcriptase enzyme, eg. Superscipt II, Gibco BRL, is followed by PCR amplification of the resultant product using specific oligonucleotide primers, for example, those previously described. The resulting amplified cDNA is ligated into an expression vector, eg. pGEMEX®-1 vector available from Promega. Competent cells, eg. bacterial strain JM109(DE3), also available from Promega, are transformed using this vector, effective transforms selected and cultured. Expression of the encoded protein is then induced with a suitable promotor, eg. IPTG, and the expressed protein purified from the cell culture using standard biochemical procedures, eg. cell lysis and polyacrylamide gel electrophoresis.

2. An alternative method for examining the functional protein encoded by the cDNA described above, is to induce transcription of the cloned cDNA, as above, and to purify the specific mRNA from the cell culture as described. The purified mRNA is introduced into competent cells, eg. frog oocytes or Chinese hamster ovary cells, and the function of the encoded protein studied by standard pharmacological and physiological techniques, eg. microelectrode recording and receptor binding techniques.

3. As 1 above, but introducing the cDNA into a coupled transcription-translation system, eg. TNT, Promega with subsequent purification and analysis of the encoded protein as described.

The invention is illustrated by the following Examples:

EXAMPLE 1

This example describes the identification and sequencing of an allelic human H_2 receptor gene using certain novel oligonucleotide primers according to the invention.

A polymerase chain reaction (PCR) product is prepared from human DNA.

DNA was extracted from human brain tissue by first pulverizing approximately 1g of tissue in liquid nitrogen then adding to 10ml lysis buffer (0.32M sucrose, 10mM Tris, 5mM magnesium chloride 1% Triton X-100 pH8.0). This solution was centrifuged (9,000 rpm 15 mins) to pellet the tissue, the lysis buffer was drawn off and the pellet resuspended in 4.5ml 75 mM sodium chloride, 24mM EDTA. This solution was then incubated for 3 hours with 250 μl 10% SDS and 2mg proteinase K at 56°C. This aqueous phase was then extracted twice with 5ml of

phenol:chloroform:isoamyl alcohol (25:24:1). Then sodium acetate to 0.3M pH7.5 and 2 volumes of ethanol (at -20°C) were added to the aqueous phase and the DNA hooked out into TE buffer. The concentration of the DNA was determined by measuring the optical density of the sample, at a wavelength of 260nm.

The DNA was then amplified by the polymerase chain reaction using the oligonucleotide primers 1) and 2) (as hereinbefore described) for 36 cycles. The timing for each cycle was as follows; 1 min at 94°C, 1.5 min at 56°C and 2 mins at 72°C, this was then followed by a 10 min extension at 72°C (Amplitaq DNA polymerase Perkin-Elmer Cetus). This reaction produced a DNA fragment of 1047 base pair when analyzed by gel electrophoresis.

Following PCR amplification of the DNA, the PCR products were immediately ligated and cloned into the TA cloning system (InvitroGen). The transformed cells were plated onto Luria-Bertani plates containing 50μl/ml amplicillin and 1.6mg X-Gal. Plates were then incubated overnight at 37°C, then moved to 4°C for 4 hours to allow for colour development. Positive (white colonies) were then analyzed by growing a 5ml culture overnight at 37°C extracting the plasmids (Qiaspin minipreps (Qiagen)) and performing an EcoRI digest to ensure the correct size product was contained in the plasmid. The plasmid used to

clone the PCR product is the pCR™II Vector, which is transformed into One Shot™ INVαF' Competent cells.

Both strands of the cloned PCR product were sequenced using the dideoxynucleotide chain-terminated method, carried out with Sequenase version 2.0 (Amersham/USB). Partial sequencing of short stretches of the cloned DNA utilised all the oligonucleotide primers 1) to 9) hereinbefore described. The cloned PCR product was shown to be identical to the gastric cDNA of Gantz et al except for the previously mentioned six single base changes.

Results and discussions

The sequence derived from the above described method is listed below and in SEQ ID NO: 2.

5'

CCAATGGCACAGCCTCTTCCTTTTGCCTGGACTCTACCGCATGCAAGATCACCAT
CACCGTGGTCCTTGCGGTCCTCATCCTCATCACCGTTGCTGGCAATGTGGTCGTC
TGTCTGGCCGTGGGCTTGAACCGCCGGCTCCGCAACCTGACCAATTGTTTCATCG
TGTCCTTGGCTATCACTGACCTGCTCCTCGGCCTCCTGGTGCTGCCCTTCTCTGC
CATCTACCAGCTGTCCTGCAAGTGGAGCTTTGGCAAGGTCTTCTGCAATATCTAC
ACCAGCCTGGATGTGATGCTCTGCACAGCCTCCATTCTTAACCTCTTCATGATCA
GCCTCGACCGGTACTGCGCTGTCATGGACCCACTGCGGTACCCTGTGCTGGTCAC
CCCAGCTCGGGTCGCCATCTCTCTGGTCTTAATTTGGGTCATCTCCATTACCCTG
TCCTTTCTGTCTATCCACCTGGGGTGGAACAGCAGGAACGAGACCAGCAAGGGCA

ATCATACCACCTCTAAGTGCAATGTCCAGGTCAATGAAGTGTACGGGCTGGTGGA
TGGGCTGGTCACCTTCTACCTCCCGCTACTGATCATGTGCATCACCTACTACCGC
ATCTTCAGGGTCGCCCGGGATCAGGCCAAGAGGATCGATCACATTAGCTCCTGGA
AGGCAGCCACCATCAGGGAGCACAGAGCCACAGTGACACTGGCCGCCGTCATGGG
GGCCTTCATCATCTGCTGGTTTCCCTACTTCACCGCGTTTGTGTACCGTGGGCTG
AGAGGGGATGATGCCATCAATGAGATGTTAGAAGCCATCGTTCTGTGGCTGGGCT
ATGCCAACTCAGCCCTGAACCCCATCCTGTATGCTGCGCTGAACAGAGACTTCCG
CACCGGGTACCAACAGCTCTTCTGCTGCAGGCTGGCCAACCGCAACTCCCACAAA
ACTTCTCTGAGGTCCAACGCCTCTCAGCTGTCCAGGACCCAAAGCCGAGAACCCA
GGCAACAGGAAGAGAAACCCCTGAAGCTCCAGGTGTGGAGTGGGACAGAAGTCACG
3'

Example 2

This example describes the confirmation of the presence of the base changes in a larger population. This is made possible by an assay based upon PCR amplification of a 909 base pair fragment of the H_2 receptor gene from human DNA, followed by cleavage utilising specific restriction endonucleases. It will apparent to those skilled in the art that single base changes could be detected using other techniques known to those in the art which include single stranded confirmational polymorphisms (sscp), chemical cleavage, PCR thermoligase reactions etc.

Samples of blood are collected from human volunteers into EDTA coated tube, 1ml of this blood is heated to

100°C for 15 minutes then spun in a microcentrifuge at 13,000Xg for 15 minutes. This supernatant is collected, and the cell debris is discarded. Then 0.5-3μl of this supernatant is utilised as template DNA for a PCR reaction to amplify a portion of the receptor gene between bases 8 and 915. The conditions for this PCR reaction are 3mM $MgCl_2$ (Gibco BRL),1X PCR buffer (Gibco BRL) 1mM of each dATP, dGTP, dGTP and dTTP (Promega) 10 pmoles of each of oligonucleotide primers 1) and 5) (hereinbefore described) and 1 unit Taq DNA polymerase (Gibco BRL), in a total volume adjusted to 50μl by sterile DNAse free water. This mix is then subjected to the following conditions; 96°C 5 minutes, then 35 cycles of 96°C for 1 minute, 56°C for 1 minute, 72°C for 1 minute and 20 seconds.

10μl of the resultant products are then analysed on a 1% agarose gel to ensure that the above reaction is correctly amplifying the target DNA fragment. Then 11.5μl of the PCR mix is added to 2 units *TaqI* restriction endonuclease (Fermentas) and 1.5μl of 10X buffer and incubated at 65°c for 3-24 hours. The products of this reaction are then analysed on a 2.5% agarose gel. If the original sequence described by Gantz (nominated H_2A) has been amplified, then bands of 574 and 335 base pairs are seen which indicates that the individual is an A/A homozygote. If the sequence described in Example 1 (nominated H_2B) has been amplified, then following the

TaqI cleavage of the PCR product , bands of 335, 306 and 268 base pairs can be seen, indicating that that individual is a B/B homozygote. If bands of 574, 335, 306 and 268 base pairs can be seen, then that individual is an A/B heterozygote.

Typical results are illustrated in Figure 1, which shows a 2.5% TBE Agarose gel stained with ethidium bromide, showing *TaqI* digestion patterns of a 909 base pair PCR fragment, from 4 separate individuals.

Lanes A + G - 100 base pair DNA marker (Gibco BRL)

Lanes B + F - Band pattern indicative of an A/B heterozygote

Lanes C + E - Band pattern indicative of an B/B homozygote

Lane D - Blank

Arrows indicated the sizes of the DNA fragments in lanes B to F.

Primers: Oswell DNA Services)

1) upstream: 5' CCAATGGCACAGCCTCTT 3'(as in SEQ ID NO:1)

2) upstream: 5' CCAGGCAACAGGAAGAGA 3'(as in SEQ ID NO:5)

5)downstream: 5' GCAGCAGAAGAGCTGTTG 3'(as in SEQ ID NO:7)

Example 3

A method as described in example 2 is applied to a series of DNA samples extracted from schizophrenic

individuals, their first degree relatives, and normal controls. There is observed a statistically significant difference of P less than 0.01 the pattern seen in the genotype of these individuals, as described in the table below:

Diagnosis	H_2 Genotype		
	A/A	A/B	B/B
Controls	12.1%	48.5%	39.4%
Schizophrenia	9.8%	26.8%	63.4%
1st degree relatives	6.1%	12.1%	81.8%

Discussion

The variable sequence is explained by a polymorphic allelic variation within the human population for the gene encoding the H_2 receptor protein. This allelic polymorphism may lead to substantial variation in the effect of activation of the encoded receptor by histamine, either in the efficacy of histamine binding, the duration of activation, or the intracellular effects of such activation. It is envisaged that such variation resulting from allelic polymorphism may underline susceptibility to specific disorders, both affecting the brain and/or involving other systems or organs. In summary, this variation in the human H_2 receptor gene and its products, including, for example, mRNA and proteins, could be used as a method of establishing individual risk

to a particular psychiatric or neurological or other illness eg. schizophrenia.

Alternative embodiments of the invention can be envisaged by those skilled in the art from the information contained herein. All such alternative embodiments are intended to lie within the scope of this application.

The reader's attention is directed to all papers and documents which are filed concurrently with this specification and which are open to public inspection with this specification, and the contents of all such papers and documents are incorporated herein by reference.

All the features disclosed in this specification (including any accompanying claims, abstract and drawings), and/or all of the steps or any method or process so disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive.

Each feature disclosed in this specification (including any accompanying claims, abstract and drawings), may be replaced by alternative features serving the same, equivalent or similar purpose, unless expressly stated otherwise. Thus, unless expressly

stated otherwise, each feature disclosed is one example only of a generic series of equivalent or similar features.

The invention is not restricted to the details of the foregoing embodiments. This invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method or process so disclosed.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: The University of Sheffield

(B) STREET: Western Bank

(C) CITY: Sheffield

(D) STATE OR PROVINCE: South Yorkshire

(E) COUNTRY: United Kingdom

(F) POSTAL CODE: S10 2TN

(A) NAME: Heath, Paul Roy (US only)

(B) STREET: 91 Abbey Lane

(C) CITY: Sheffield

(D) STATE OR PROVINCE: South Yorkshire

(E) COUNTRY: United Kingdom

(F) POSTAL CODE: S8 0BR

(A) NAME: Orange, Paul Richard (US only)

(B) STREET: 56 Salisbury Road, Crookes,

(C) CITY: Sheffield

(D) STATE OR PROVINCE: South Yorkshire

(E) COUNTRY: United Kingdom

(F) POSTAL CODE: S10 1WB

(A) NAME: Pearson, Ronald Carl Alan (US only)

(B) STREET: 12 Kingswood Road, Jesmond,

(C) CITY: Newcastle-upon-Tyne

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(E) COUNTRY: United Kingdom

(F) POSTAL CODE: NE2 3NS

(A) NAME: Wright, Simon Ralph (US only)

(B) STREET: 56 Hunter Hill Road, Hunter's Bar

(C) CITY: Sheffield

(D) STATE OR PROVINCE: South Yorkshire

(E) COUNTRY: United Kingdom

(F) POSTAL CODE: S11 8UE

(ii) TITLE OF INVENTION: Improvements in or Relating to the Detection of Variations in Human Histamine H_2 Receptors

(iii) NUMBER OF SEQUENCES: 11

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Dibb Lupton Broomhead

(B) STREET: Fountain Precinct, Balm Green

(C) CITY: Sheffield

(D) STATE OR PROVINCE: South Yorkshire

(E) COUNTRY: United Kingdom

(F) POSTAL CODE: S1 1RZ

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy Disc 1.44 MB DSHD

(B) COMPUTER: IBM PC Compatible

(C) OPERATING SYSTEM: MS-DOS 6.22

(D) SOFTWARE: Word Perfect 5.1 for DOS

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NO: Not known

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Hall, Robert Leonard

(B) REGISTRATION NO:

(C) REFERENCE/DOCKET NUMBER: P31409PC

(ix) TELECOMMUNCATION INFORMATION:

(A) TELEPHONE: 0114 283 3253

(B) TELEFAX: 0114 273 0312

(C) TELEX: ------

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 413 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double stranded

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: homo sapiens

(C) INDIVIDUAL/ISOLATE: human tissue sample no:S113

(D) DEVELOPMENT STAGE: fully mature

(F) TISSUE TYPE: brain

(vii) IMMEDIATE SOURCE:

(B) CLONES: ONESHOT™ INVαF' clone P01

(ix) FEATURES:

(A) NAME/KEY: Taq I restriction endonuclease site

(B) LOCATION: bases 253-257

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
CA GCT CGG GTC GCC ATC TCT CTG GTC TTA ATT TGG GTC ATC TCC ATT  47
   Ala Arg Val Ala Ile Ser Leu Val Leu Ile Trp Val Ile Ser Ile
           135                 140                 145

ACC CTG TCC TTT CTG TCT ATC CAC CTG GGG TGG AAC AGC AGG AAC GAG 95
Thr Leu Ser Phe Leu Ser Ile His Leu Gly Trp Asn Ser Arg Asn Glu
        150                 155                 160
```

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ACC AGC AAG GGC AAT CAT ACC ACC TCT AAG TGC AAT GTC CAG GTC AAT 143
Thr Ser Lys Gly Asn His Thr Thr Ser Lys Cys Asn Val Gln Val Asn
    165                 170                 175

GAA GTG TAC GGG CTG GTG GAT GGG CTG GTC ACC TTC TAC CTC CCG CTA 191
Glu Val Tyr Gly Leu Val Asp Gly Leu Val Thr Phe Tyr Leu Pro Leu
180                 185                 190                 195

CTG ATC ATG TGC ATC ACC TAC TAC CGC ATC TTC AGG GTC GCC CGG GAT 239
Leu Ile Met Cys Ile Thr Tyr Tyr Arg Ile Phe Arg Val Ala Arg Asp
                200                 205                 210

CAG GCC AAG AGG ATC GAT CAC ATT AGC TCC TGG AAG GCA GCC ACC ATC 287
Gln Ala Lys Arg Ile Asp His Ile Ser Ser Trp Lys Ala Ala Thr Ile
            215                 220                 225

AGG GAG CAC AGA GCC ACA GTG ACA CTG GCC GCC GTC ATG GGG GCC TTC 335
Arg Glu His Arg Ala Thr Val Thr Leu Ala Ala Val Met Gly Ala Phe
        230                 235                 240

ATC ATC TGC TGG TTT CCC TAC TTC ACC GCG TTT GTG TAC CGT GGG CTG 383
Ile Ile Cys Trp Phe Pro Tyr Phe Thr Ala Phe Val Tyr Arg Gly Leu
    245                 250                 255

AGA GGG GAT GAT GCC ATC AAT GAG ATG TTA                         413
Arg Gly Asp Asp Ala Ile Asn Glu Met Leu
260                 265
```

(3) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1046 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double stranded

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic dna

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: homo sapiens

(C) INDIVIDUAL/ISOLATE: human tissue sample no:S113

(D) DEVELOPMENT STAGE: fully mature

(F) TISSUE TYPE: brain

(vii) IMMEDIATE SOURCE:

(B) CLONES: ONESHOT™ INVαF' clone P01

(ix) FEATURES:

(A) NAME/KEY: Taq I restriction endonuclease sites

(B) LOCATION: bases 640-644 and 334-337

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
CC AAT GGC ACA GCC TCT TCC TTT TGC CTG GAC TCT ACC GCA TGC AAG  47
   Asn Gly Thr Ala Ser Ser Phe Cys Leu Asp Ser Thr Ala Cys Lys
       5                   10                  15

ATC ACC ATC ACC GTG GTC CTT GCG GTC CTC ATC CTC ATC ACC GTT GCT 95
Ile Thr Ile Thr Val Val Leu Ala Val Leu Ile Leu Ile Thr Val Ala
    20                  25                  30

GGC AAT GTG GTC GTC TGT CTG GCC GTG GGC TTG AAC CGC CGG CTC CGC 143
Gly Asn Val Val Val Cys Leu Ala Val Gly Leu Asn Arg Arg Leu Arg
35                  40                  45                  50

AAC CTG ACC AAT TGT TTC ATC GTG TCC TTG GCT ATC ACT GAC CTG CTC 191
Asn Leu Thr Asn Cys Phe Ile Val Ser Leu Ala Ile Thr Asp Leu Leu
                55                  60                  65

CTC GGC CTC CTG GTG CTG CCC TTC TCT GCC ATC TAC CAG CTG TCC TGC 239
Leu Gly Leu Leu Val Leu Pro Phe Ser Ala Ile Tyr Gln Leu Ser Cys
            70                  75                  80

AAG TGG AGC TTT GGC AAG GTC TTC TGC AAT ATC TAC ACC AGC CTG GAT 287
Lys Trp Ser Phe Gly Lys Val Phe Cys Asn Ile Tyr Thr Ser Leu Asp
        85                  90                  95

GTG ATG CTC TGC ACA GCC TCC ATT CTT AAC CTC TTC ATG ATC AGC CTC 335
Val Met Leu Cys Thr Ala Ser Ile Leu Asn Leu Phe Met Ile Ser Leu
    100                 105                 110
```

30

GAC CGG TAC TGC GCT GTC ATG GAC CCA CTG CGG TAC CCT GTG CTG GTC 383
Asp Arg Tyr Cys Ala Val Met Asp Pro Leu Arg Tyr Pro Val Leu Val
115 120 125 130

ACC CCA GCT CGG GTC GCC ATC TCT CTG GTC TTA ATT TGG GTC ATC TCC 431
Thr Pro Ala Arg Val Ala Ile Ser Leu Val Leu Ile Trp Val Ile Ser
135 140 145

ATT ACC CTG TCC TTT CTG TCT ATC CAC CTG GGG TGG AAC AGC AGG AAC 479
Ile Thr Leu Ser Phe Leu Ser Ile His Leu Gly Trp Asn Ser Arg Asn
150 155 160

GAG ACC AGC AAG GGC AAT CAT ACC ACC TCT AAG TGC AAT GTC CAG GTC 527
Glu Thr Ser Lys Gly Asn His Thr Thr Ser Lys Cys Asn Val Gln Val
165 170 175

AAT GAA GTG TAC GGG CTG GTG GAT GGG CTG GTC ACC TTC TAC CTC CCG 575
Asn Glu Val Tyr Gly Leu Val Asp Gly Leu Val Thr Phe Tyr Leu Pro
180 185 190

CTA CTG ATC ATG TGC ATC ACC TAC TAC CGC ATC TTC AGG GTC GCC CGG 623
Leu Leu Ile Met Cys Ile Thr Tyr Tyr Arg Ile Phe Arg Val Ala Arg
195 200 205 210

GAT CAG GCC AAG AGG ATC GAT CAC ATT AGC TCC TGG AAG GCA GCC ACC 671
Asp Gln Ala Lys Arg Ile Asp His Ile Ser Ser Trp Lys Ala Ala Thr
215 220 225

ATC AGG GAG CAC AGA GCC ACA GTG ACA CTG GCC GCC GTC ATG GGG GCC 719
Ile Arg Glu His Arg Ala Thr Val Thr Leu Ala Ala Val Met Gly Ala
230 235 240

TTC ATC ATC TGC TGG TTT CCC TAC TTC ACC GCG TTT GTG TAC CGT GGG 767
Phe Ile Ile Cys Trp Phe Pro Tyr Phe Thr Ala Phe Val Tyr Arg Gly
245 250 255

CTG AGA GGG GAT GAT GCC ATC AAT GAG ATG TTA GAA GCC ATC GTT CTG 815
Leu Arg Gly Asp Asp Ala Ile Asn Glu Met Leu Glu Ala Ile Val Leu
260 265 270

TGG CTG GGC TAT GCC AAC TCA GCC CTG AAC CCC ATC CTG TAT GCT GCG 863
Trp Leu Gly Tyr Ala Asn Ser Ala Leu Asn Pro Ile Leu Tyr Ala Ala
275 280 285 290

CTG AAC AGA GAC TTC CGC ACC GGG TAC CAA CAG CTC TTC TGC TGC AGG 911
Leu Asn Arg Asp Phe Arg Thr Gly Tyr Gln Gln Leu Phe Cys Cys Arg
295 300 305

CTG GCC AAC CGC AAC TCC CAC AAA ACT TCT CTG AGG TCC AAC GCC TCT 959
Leu Ala Asn Arg Asn Ser His Lys Thr Ser Leu Arg Ser Asn Ala Ser
310 315 320

CAG CTG TCC AGG ACC CAA AGC CGA GAA CCC AGG CAA CAG GAA GAG AAA 1007
Gln Leu Ser Arg Thr Gln Ser Arg Glu Pro Arg Gln Gln Glu Glu Lys
325 330 335

CCC CTG AAG CTC CAG GTG TGG AGT GGG ACA GAA GTC ACG 1046
Pro Leu Lys Leu Gln Val Trp Ser Gly Thr Glu Val Thr
340 345 350

(4) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single stranded
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic oligonucleotide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CCAATGGCAC AGCCTCTT 18

(5) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single stranded

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic oligonucleotide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CGTGACTTCT GTCCCACT 18

(6) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single stranded

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic oligonucleotide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CCAGGCAACA GGAAGAGA 18

(7) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single stranded

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic oligonucleotide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

TCTCTTCCTG TTGCCTGG 18

(8) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single stranded

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic oligonucleotide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GCAGCAGAAG AGCTGTTG 18

(9) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single stranded

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic oligonucleotide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TCCAGGTCAA TGAAGTGT 18

(10) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single stranded

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic oligonucleotide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

ACACTTCATT GACCTGGA 18

(11) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single stranded

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic oligonucleotide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CCAAGAGGAT CAATCACA 18

(12) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single stranded

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic oligonucleotide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

TGTGATTGAT CCTCTTGG 18

CLAIMS

1. A nucleotide sequence coding for a region of a human histamine H_2 receptor, comprising one or more of the following base substitutions compared with the published sequence in Gantz et al (1991) Biochem Biophys Res Comm 178, 3, 1386 - 1392, and from which the positional notation is taken:

site of change	base
398	C
525	T
620	G
649	G
692	G
802	A

2. A nucleotide sequence coding for a region of a human histamine H_2 receptor, comprising the sequence (as also listed in SEQ ID NO: 1):

CAGCTCGGGTCGCCATCTCTCTGGTCTTAATTTGGGTCATCTCCATTACC
CTGTCCTTTCTGTCTATCCACCTGGGGTGGAACAGCAGGAACGAGACCAG
CAAGGGCAATCATACCACCTCTAAGTGCAATGTCCAGGTCAATGAAGTGT
ACGGGCTGGTGGATGGGCTGGTCACCTTCTACCTCCCGCTACTGATCATG
TGCATCACCTACTACCGCATCTTCAGGGTCGCCCGGGATCAGGCCAAGAG
GATCGATCACATTAGCTCCTGGAAGGCAGCCACCATCAGGGAGCACAGAG
CCACAGTGACACTGGCCGCCGTCATGGGGGCCTTCATCATCTGCTGGTTT
CCCTACTTCACCGCGTTTGTGTACCGTGGGCTGAGAGGGGATGATGCCAT
CAATGAGATGTTA

3. A nucleotide sequence coding for a region of a human histamine H_2 receptor, comprising the sequence (as also listed in SEQ ID NO: 2):

5'

CCAATGGCACAGCCTCTTCCTTTTGCCTGGACTCTACCGCATGCAAGATC
ACCATCACCGTGGTCCTTGCGGTCCTCATCCTCATCACCGTTGCTGGCAA
TGTGGTCGTCTGTCTGGCCGTGGGCTTGAACCGCCGGCTCCGCAACCTGA
CCAATTGTTTCATCGTGTCCTTGGCTATCACTGACCTGCTCCTCGGCCTC
CTGGTGCTGCCCTTCTCTGCCATCTACCAGCTGTCCTGCAAGTGGAGCTT
TGGCAAGGTCTTCTGCAATATCTACACCAGCCTGGATGTGATGCTCTGCA
CAGCCTCCATTCTTAACCTCTTCATGATCAGCCTCGACCGGTACTGCGCT
GTCATGGACCCACTGCGGTACCCTGTGCTGGTCACCCCAGCTCGGGTCGC
CATCTCTCTGGTCTTAATTTGGGTCATCTCCATTACCCTGTCCTTTCTGT
CTATCCACCTGGGGTGGAACAGCAGGAACGAGACCAGCAAGGGCAATCAT
ACCACCTCTAAGTGCAATGTCCAGGTCAATGAAGTGTACGGGCTGGTGGA
TGGGCTGGTCACCTTCTACCTCCCGCTACTGATCATGTGCATCACCTACT
ACCGCATCTTCAGGGTCGCCCGGGATCAGGCCAAGAGGATCGATCACATT
AGCTCCTGGAAGGCAGCCACCATCAGGGAGCACAGAGCCACAGTGACACT
GGCCGCCGTCATGGGGGCCTTCATCATCTGCTGGTTTCCCTACTTCACCG
CGTTTGTGTACCGTGGGCTGAGAGGGGATGATGCCATCAATGAGATGTTA
GAAGCCATCGTTCTGTGGCTGGGCTATGCCAACTCAGCCCTGAACCCCAT
CCTGTATGCTGCGCTGAACAGAGACTTCCGCACCGGGTACCAACAGCTCT
TCTGCTGCAGGCTGGCCAACCGCAACTCCCACAAAACTTCTCTGAGGTCC
AACGCCTCTCAGCTGTCCAGGACCCAAAGCCGAGAACCCAGGCAACAG
GAAGAGAAACCCCTGAAGCTCCAGGTGTGGAGTGGGACAGAAGTCACG

3'

4. A protein and/or a peptide derived from a DNA comprising a nucleotide sequence according to any one of Claims 1 to 3.

5. An expression or recombinant vector comprising a nucleotide sequence coding for a protein or a peptide according to Claim 4.

6. Oligonucleotides, suitable for use as primers for the amplification or sequencing of DNA corresponding to a region of a human histamine H_2 receptor, selected from:

1) 5' CCAATGGCACAGCCTCTT 3' (also listed in SEQ ID NO: 3)
2) 5' CGTGACTTCTGTCCCACT 3' (also listed in SEQ ID NO: 4)
3) 5' CCAGGCAACAGGAAGAGA 3' (also listed in SEQ ID NO: 5)
4) 5' TCTCTTCCTGTTGCCTGG 3' (also listed in SEQ ID NO: 6)
5) 5' GCAGCAGAAGAGCTGTTG 3' (also listed in SEQ ID NO: 7)
6) 5' TCCAGGTCAATGAAGTGT 3' (also listed in SEQ ID NO: 8)
7) 5' ACACTTCATTGACCTGGA 3' (also listed in SEQ ID NO: 9)

8) 5' CCAAGAGGATCAATCACA 3' (also listed in SEQ ID NO: 10)

9) 5' TGTGATTGATCCTCTTGG 3' (also listed in SEQ ID NO: 11)

7. The use, as a primer for the amplification or sequencing of DNA corresponding to a region of a human histamine H_2 receptor, of an oligonucleotide selected from:

1) 5' CCAATGGCACAGCCTCTT 3' (also listed in SEQ ID NO: 3)

2) 5' CGTGACTTCTGTCCCACT 3' (also listed in SEQ ID NO: 4)

3) 5' CCAGGCAACAGGAAGAGA 3' (also listed in SEQ ID NO: 5)

4) 5' TCTCTTCCTGTTGCCTGG 3' (also listed in SEQ ID NO: 6)

5) 5' GCAGCAGAAGAGCTGTTG 3' (also listed in SEQ ID NO: 7)

6) 5' TCCAGGTCAATGAAGTGT 3' (also listed in SEQ ID NO: 8)

7) 5' ACACTTCATTGACCTGGA 3' (also listed in SEQ ID NO: 9)

8) 5' CCAAGAGGATCAATCACA 3' (also listed in SEQ ID NO: 10)

9) 5' TGTGATTGATCCTCTTGG 3' (also listed in SEQ ID NO: 11)

8. A process for the detection of a nucleotide sequence coding for a region of a human H_2 receptor, or of cDNA corresponding to transcription products thereof, in a biological sample thereof, which comprises

a) the amplification of the DNA with at least a pair of primers selected from the oligonucleotides of Claim 6

b) the detection of the amplified sequences on an electrophoretic gel.

9. A diagnostic kit comprising at least one oligonucleotide according to Claim 6.

10. A diagnostic kit according to Claim 9 , which also comprises one or more specific restriction endonucleases capable of demonstrating one or more of the single base substitutions hereinbefore described.

11. A method for the detection of one or more inherited or acquired allelic polymorphic variations in a sample of a DNA encoding the human H_2 receptor comprising one or more of the six base

substitutions hereinbefore defined, which comprises PCR amplification of a DNA sequence.

12. A method according to Claim 11 wherein the step of PCR Amplification is followed by the step of detecting single base variations in the human H_2 receptor.

13. A method according to Claim 11 or 12 wherein the step of PCR Amplification is followed by specific restriction endonuclease digestion.

14. The use of at least one oligonucleotide according to Claim 6, for determining the base variations in the human H_2 receptor of an individual for providing information relating to disease susceptibility, disease course, prognosis and/or response to treatment.

15. The use of a nucleotide sequence according to Claim 1, 2 or 3 to derive oligoneucleotides for use in determining any of the base variations in Claim 1 for providing information relating to disease susceptibility, disease course, prognosis and/or response to treatment in an individual.

16. A nucleotide sequence, or a protein or peptide derived from a cDNA comprising the nucleotide sequence, substantially as hereinbefore described.

17. An oligonucleotide suitable for use as a primer for the amplification of DNA corresponding to a region of a human H_2 receptor substantially as hereinbefore described.

18. A process according to Claim 8, substantially as described in the Examples.

19. A host cell containing an expression or recombinant vector according to Claim 5.

FIGURE 1